FABRICATION OF GLUCOSE SENSOR USING GRAPHENE

CHONG SOO LING

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ABSTRACT

Graphene is a one-atom-thick allotrope of carbon. Due to its unique mechanical and electronic properties, graphene has been touted as very promising material for a great number of applications. In particular, biosensor is a device for the detection of specific analyte which combines a biological compound with a physicochemical detector component. There are three major components that complete a biosensor, namely the sensitive biological element, the transducer or detector element and also the associated electronics or signal processors. The working principle of graphene-based enzymatic electrodes is based on the direct electrochemistry of enzymes whereby electron transfer occurs between the electrode and the active centre of the enzymes without the participation of any mediators or reagents. In the current work, a graphene based glucose biosensor was prepared. The reduced graphene oxide (RGO)-glucose oxidase (GOx) was prepared from graphite powder as starting material. Material synthesis involved pre-oxidation of graphite to graphite oxide followed by further oxidation to graphene oxide. Subsequently, hydrazine monohydrate was used to reduce the graphene oxide to reduced graphene oxide. Finally, glassy carbon electrode (GCE) /RGO-GOx based glucose sensor was fabricated. Thereafter, the sample was tested with different concentrations of glucose solution. It was also being subjected to ultraviolet-visible absorption spectrophotometry (UV-Vis), Fourier transform infrared spectroscopy (FTIR) and cyclic voltammetry (CV). From this study, the obtained results are allowed for assessment of an optimum and sensitivity of the GCE/RGO-GOx based glucose biosensor towards the glucose concentration. It can be concluded that GCE/RGO-GOx based glucose biosensor can detect circa 3 to 7 mM glucose.

ABSTRAK

Graphene adalah alotrop satu atom -tebal karbon. Dengan ciri-ciri yang unik mekanikal dan elektronik, graphene telah dipromosikan sebagai bahan dalam sejumlah besar aplikasi. Khususnya, biosensor adalah alat untuk mengesan analit tertentu yang menggabungkan sebatian biologi dengan komponen pengesan fizikokimia . Terdapat tiga komponen utama yang melengkapkan biosensor iaitu unsur biologi sensitif, transduser atau pengesan unsur dan juga pemproses elektronik atau isyarat yang berkaitan. Prinsip kerja berasaskan graphene - elektrod enzim adalah berdasarkan kepada elektrokimia langsung enzim mana pemindahan elektron berlaku antara elektrod dan pusat aktif enzim tanpa penyertaan mana-mana pengantara atau reagen. Dalam kerja-kerja semasa, graphene berasaskan glukosa biosensor akan disediakan. Glukosa biosensor yang berasaskan konsept pengurangan graphene oksida (RGO)-glukosa oksidase (GOD) akan disediakan daripada serbuk grafit sebagai bahan permulaan . Sintesis bahan akan melibatkan pra- pengoksidaan grafit untuk grafit oksida diikuti dengan pengoksidaan lanjut untuk graphene oksida. Selepas itu, hidrazin monohydrate akan digunakan untuk menurunkan oksida graphene untuk graphene. Akhir sekali, glukosa biosensor GCE/RGO-GOx akan direka. Selepas itu, sampel akan diuji dengan kepekatan larutan glukosa. Ia juga akan tertakluk kepada ultraungu- dilihat penyerapan spektrofotometri , spektroskopi dan voltammetri berkitar. Dalam kajian ini, keputusan yang diperolehi akan membolehkan untuk penilaian sensitiviti optimum dan glukosa biosensor GCE/RGO-GOx berdasarkan kepekatan glukosa. Kajian ini menjangka bahawa GCE/RGO-GOx berasaskan glukosa biosensor boleh mengesan sekitar 3mM glukosa.

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GCE	Glassy carbon electrode
GO	Graphene oxide
GOx	Glucose oxidase
HRGO	Hydrazine reduced graphene oxide
PBS	Phosphate buffer saline
RGO	Reduced graphene oxide

1 INTRODUCTION

1.1 Background of Study

Diabetes is increasing worldwide at an unprecedented pace and has become a serious health concern during the last two decades. It is a major cause of mortality in the age group of 20–79 years. Based on its rapidly increasing incidence, it has been declared a global epidemic by the World Health Organization (WHO) The metabolic disorder in the form of diabetes mellitus can cause the deficiency of insulin and hyperglycemia. The syndrome is typically reflected by blood glucose concentration that will show readings above normal range of 4.4-6.6 mM. Diabetes is a disease that can cause fatality if left untreated (Wang J., 2008). Therefore, the diagnosis and disease management require close monitoring of blood glucose levels. In this respect, glucose sensor, functions via breaking the glucose using enzyme, is the most common biosensor applied in blood glucose level testing (News Medical; Yoo & Lee, 2010)

Graphene is a one-atom-thick allotrope of carbon. It is tightly packed into a two dimensional (2D) honeycomb lattice and is a basic building block for graphitic materials of all other dimensionalities. It can be wrapped into zero dimensional (0D) fullerens, rolled into one dimensional (1D) carbon nanotubes, or stacked into three dimensional (3D) graphite (Geim & Novoselov, 2007). Recently graphene had emerged as an interesting material in a great number of applications. This can be attributed to its unique mechanical and electronic properties (Huang, 2010). More so, there are scarcities of studies into the use of graphene as a biosensor. Most of the prior graphene studies are related to its electronic properties and applications that are confined to gas and pH sensors (Hong *et al.,* 2009).

Therefore, in this study, graphene will be employed as material of choice for fabrication of glucose sensor. From this work, it is envisaged that a functionalized graphene sheets-based bio-nanocomposite film will be developed and its application for sensitive glucose sensing will be demonstrated.

1.2 Problem Statement and Motivation

At present, the glucose sensor available in the biosensor market has several problems. It is costly and time consuming to calibrate the accurate blood glucose level for diabetes mellitus patients. Most of the current glucose sensor employed metal electrodes such as platinum (Pt) and gold (Au) which made the materials cost increased. Besides that, the enzymatic glucose biosensor has a short life span.

To overcome these problems, a recently promising material which is graphene is employed in the research to develop a glucose sensor which it is cheaper compared to those existing materials gold and platinum as the electrode inside the biosensor. Graphene has the high conductivity and is a good semiconductor which can be reliable to use as a raw material to develop a glucose biosensor.

1.3 Objective

i. To develop a glucose sensor.

ii. To quantify the amount of glucose solution that can be detected by the developed graphene based glucose sensor.

1.4 Scope

To achieve the aforementioned objectives, the current scopes of research have been outlined:

i. Fabrication of glucose sensor using graphene

   To model the degree of connection between glucose and the graphene based sensor and how the graphene based sensor detect the glucose level.

ii. Quantification of the range of glucose level detection of graphene based glucose sensor

   To quantify the concentration of glucose solution that can be detected by graphene based glucose sensor according to human blood glucose level which is 4.4 to 6.6 mM.

2 LITERATURE REVIEW

2.1 Diabetes Mellitus

2.1.1 History of Diabetes Mellitus

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism causing from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. There are three major types of diabetes which are type 1 diabetes, type 2 diabetes and gestational diabetes (WebMD Site).

Type 1 diabetes is used to be called as juvenile-onset diabetes because it often begins in childhood. It is also called as insulin-dependent diabetes. It caused by the body attacking its own pancreas with antibodies and also is an autoimmune condition. Normally the pancreas of people with type 1 diabetes is damaged and doesn't make insulin. It could cause by genetic predisposition. The faulty beta cells in the pancreas that normally produce insulin also may be result in (Darwiche *et al.,* 2012)

The most common form of diabetes is type 2 diabetes. In type 2 diabetes, the body can't obtain the glucose well because either the bodydoes not produce enough insulin or the cells ignore the insulin. Insulin is necessary for the body to convert glucose for energy (Frank *et al.,* 2001).

Gestational diabetes is normally found in pregnant women. Pregnant women who have never had diabetes before but who have high blood glucose are said to have gestational diabetes. Based on recently announced diagnostic criteria for gestational diabetes, it is estimated that gestational diabetes affects 18% of pregnancies (Sacks, 2011). Gestational diabetes starts when your body is not able to make and use all the insulin it needs for pregnancy. Without enough insulin, glucose cannot leave the blood and be changed to energy. Glucose builds up in the blood to high levels. This is called hyperglycemia.

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.

Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin.

2.1.2 Current Methods of Glucose Level Detection for Diabetes Mellitus

Previously the people with diabetes mellitus usually using an accurate method of measuring blood glucose concentrations by detecting both hyperglycaemia and hypoglycaemia. There are many types of glucose screening testing. One of those testing is fasting plasma glucose (FPG) which also a component of diagnostic testing. Fasting is defined as no food and beverage consumption other than water for at least 8 h before testing. FPG is a carbohydrate metabolism test which measures blood sugar levels and is used to diagnose diabetes. Relatively simple and inexpensive, the test exposes problems with insulin functioning (Baker, 2013). The FPG test is normally performed in clinical as it is easier and faster to perform, more convenient and acceptable to patients, and much cheaper. An FPG $\geq$ 126 mg/dL is an indication for retesting, which ought to be repeated on a different day to confirm a diagnosis. Oral glucose tolerance test (OGTT) is another diabetes test comes after FPG. When FPG is < 126 mg/dL and there is a high suspicion

for diabetes, an OGTT should be carrying out (Schneider *et al.*, 2003). OGTT is testing the blood glucose level before and after intake of glucose solution which normally is 75 g of glucose. The objective of undergo OGTT is to determine the ability of metabolising intake of sugar or carbohydrate of the body.

Table 2-1: Diabetes Mellitus and Prediabetic states as defined by the FPG and OGTT (75 g anhydrous glucose) (Schneider *et al.*, 2003).

Fasting Plasma Glucose	2 h post OGTT Plasma Glucose		Interpretation
< 6.1 mmol/L	And	< 7.8 mmol/L	No diabetes
6.1 – 6.9 mmol/L	And	< 7.8 mmol/L	Impaired fasting glycaemia
< 7.0 mmol/L	And	7.8-11.0 mmol/L	Impaired glucose tolerance
≥ 7.0 mmol/L	And/Or	11.1 mmol/L	Consistent with diabetes mellitus

Continuous glucose monitoring system (CGMS) is also another blood glucose level testing for diabetes mellitus patients. It is a U.S. Food and Drug Administration (FDA) - approved device that records blood sugar levels throughout the day and night. (Vashist, 2013) The mechanism of CGMS is a tiny glucose sensing device also named as sensor inserted under the skin of the abdomen. The sensor measures the level of glucose in the tissue every 10 seconds and sends the information via a wire to a cell phone-sized device called a "monitor" that you attach to a belt or the waistline of your pants. The system automatically records an average glucose value every five minutes for up to seven days. The results of at least four fingers stick blood sugar readings taken with a standard glucose meter and taken at different times each day are entered into the monitor for calibration (Schulman *et al.*, 1996). The main advantage of continuous glucose monitoring is that it can help identify fluctuations and trends that would otherwise go unnoticed with standard glycated haemoglobin (HbA1c) tests and intermittent finger stick measurements.

In both of these glucose level testing methods, glucose sensor is applied in their mechanism. There are three types of glucose sensors available in the current market, which were developed based on different technology platforms. The first type of glucose sensor is a classical amperometric sensor using thick film technology. The second type is a fiber-optic fluorometric glucose sensor based on oxygen measurement and third type is a spectroscopic glucose sensor using mid-infrared spectroscopy (CLINICIP, 2004).

Amperometric glucose sensor is an enzymatic- electrochemical sensor. It was developed for CGMS based on a novel miniaturized planar sensor flow-through cell arrangement. The sensor, which was manufactured using polymer thick film technology, features four electrodes serving as the amperometric detection unit and for measuring conductivity. There a biocompatible selective diffusion barrier to protect the electrode surfaces and the enzyme immobilisate against interfering bio-compounds from the body fluid (CLINICIP, 2004). The mechanism of amperometric sensors is employing an enzyme immobilized on the top of the working electrode (Wilson & Gifford , 2005). For example Clark-based amperometric sensors employ a glucose oxidase enzyme (GOD) then flavin adenine dinucleotide redox cofactor of GOD catalyzes the oxidation of glucose to glucarolactone, as shown in Equations (1) and (2):

$$Glucose + GOD(FAD) \rightarrow Glucolactone + GOD(FADH_2) \quad (1)$$

$$GOD(FADH_2) + O_2 \rightarrow GOD(FAD) + H_2O_2 \quad (2)$$

The generated H_2O_2 is amperometrically assessed on the surface of working electrode by the application of the suitable redox potential, hence relating the current to glucose concentration (Malitesta *et al.*, 1990),

$$H_2O_2 \overset{+v}{\rightarrow} O_2 + 2e^- + H^+ \quad (3)$$

A fiber-optic biosensor for glucose concentration detection has been designed, based on electrostatic self-assembly. The fibre-optic sensor system uses the enzyme-based oxidation of glucose, in combination with an optical oxygen sensor as transducer. A fibre-optic dual sensor setup was integrated into a flow-through cell. One sensor measures oxygen sensor only, while the second oxygen sensor is covered with an enzyme

layer (Bennett *et al.*, 2006). The fluorescence of decacyclene (ex 385 nm, em 450–600 nm) is quenched by oxygen. The ruthenium complex, tris(1,10-phenanthrolene)ruthenium chloride (ex 447 nm, em 604 nm), has also been used as an oxygen detector in enzymatic-based glucose sensors by several groups, oxygen quenching the fluorescence of the ruthenium compound (Trettnak *et al.*, 1988). The major advantage of this approach is the excellent selectivity of the oxygen optode transducer. The sensors have already been tested in both clinical studies and intensive care units with very promising results. Improvements of the transducers are currently underway, including the synthesis of new fluorophors with improved properties such as greater brightness and lower temperature dependence.

Spectroscopic glucose sensor is a non-invasive optical technique to detect the glucose level. Existing non-invasive optical techniques include near infrared spectroscopy, Raman spectroscopy, photo-acoustic spectroscopy, femtosecond pulse interferometry, optical coherence tomography, and different types of fluorescence (Wang *et al.*, 2008). The working principle of it is measuring the absorption of light at certain wavelength of relectance or transmittance in the blood solution. A non-invasive analysis of absorption ratio is carried out for different sets of the wavelengths. Changes in the detected reflectance or transmittance ratios are then correlated with specific material properties, such as the concentration of glucose in a subject's circulatory system (Shao *et al.*, 2012).

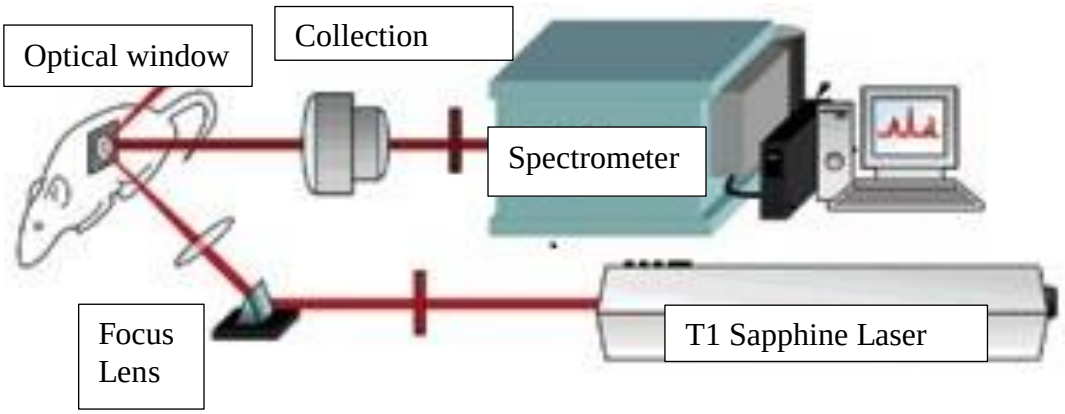

Figure 2-1: The process of detecting a rat blood glucose level by using spectroscopic glucose sensor (Biophotonics).

Table 2-2: Comparison of current blood glucose level detection methods.

Glucose level detection method	Working Principle	Sensitivity	References
Fasting plasma glucose	Carbohydrate metabolism test by testing the plasma glucose.	68%	Baker, 2013
Continuous glucose monitoring system	A tiny glucose sensing device inserted under the skin of the abdomen.	79%	Schulman et al., 1996
Amperometric glucose sensor	Enzymatic-electrochemical sensor	-	Wilson & Gifford , 2005
Fiber-optic fluorometric glucose sensor	Combination of enzyme-based oxidation of glucose with an optical oxygen sensor as transducer.	-	Bennett *et al.*, 2006
Spectroscopic glucose sensor	Measuring the absorption of light at certain wavelength of relectance or transmittance in the blood solution.	75%	Shao *et al.*, 2012

2.2 Graphene

2.2.1 History of Graphene

Nearly 500 years, graphite has been known as a full and naturally mineral. B.C. Brodie, a British scientist was the first graphene oxide explorer in the early of 1859. B.C. Brodie was interested in the molecular formula of ‘‘graphite’’ and its discrete molecular weight. The oxidation of graphite by employed potassium chloride ($KClO_3$) into slurry of graphite in fuming nitric acid (HNO_3) was first proposed by him and named as Brodie's method (Wang & Hu, 2011). After 40 years, L .Staudenmaier another scientist modified Brodie's method. He replaced nitric acid with a mixture of sulphuric acid and nitric acid to increase the acidity of the reactants. Staudenmaier's method is not applicable because it is hazardous and time-consuming (Gao, Graphite Oxide: Structure, Reduction and Applications, 2012). After 60 years of Staudenmaier's strategy, chemists Hummers and Offeman in Mellon Institution of Industrial Research developed a different method for synthesis graphene oxide. Hummers' method is less hazardous oxidation process by used potassium permanganate ($KMnO_4$) instead of potassium chlorate ($KClO_3$) as oxidizing agent (Wang & Hu, 2011). Potassium chlorate ($KClO_3$) is flammable chemical oxidizing agents, so it is hazardous for the environment whereas potassium permanganate ($KMnO_4$) has none of flammable characteristics. The whole oxidation process of Hummers' method can finish within 2 h and final products have a higher degree of oxidation than Staudenmaier's product. However, Hummers' method usually incompletely oxidized graphite core with graphene oxide shells (Gao, 2012), so Kovtyukhova done some modification of Hummers' method in 1999. Modified Hummers' method was adopted to synthesize graphite oxide. The purpose of the modification was to assist graphite to achieve a higher degree of oxidation by pre-oxidation.

Graphene in strictly two-dimensional (2D) crystals are known as not exist materials which argued by Landau and Peirels due to its thermodymically unstable. This argument was then strongly supported by Mermin through numerous of experimental observations. Throughout one of the experiment showed that graphene in dozens of atomic layers form become unstable when the melting temperature of the thin film decreases gradually together with the thickness. Due to this reason, atomic monolayer was known only as integral part of the three-dimensional (3D) structure where it only can grow on the top of

monocrystal layer with matching crystal lattice (Geim & Novoselov, 2007). In 2005, Professor Andre Geim's group first studied the experimentally temperature quantum hall effect on a real piece of graphene, which was obtained by mechanical exfoliation of Highly Oriented Pyrolytic Graphite (HOPG). In 2010, Andre Geim and Konstantin Novoselov won the 2010 Nobel Prize in Physics due to discovery of graphene (Geim A. , 2011).

2.2.2 Properties of Graphene

Graphene is a two-dimensional sheet of sp2-hybridized carbon. Graphene is a one-atom-thick allotrope of carbon. It is tightly packed into a two dimensional (2D) honeycomb lattice and is a basic building block for graphitic materials of all other dimensionalities. It can be wrapped into zero dimensional (0D) fullerences, rolled into one dimensional (1D) carbon nanotubes, or stacked into three dimensional (3D) graphite (Geim & Novoselov, 2007).

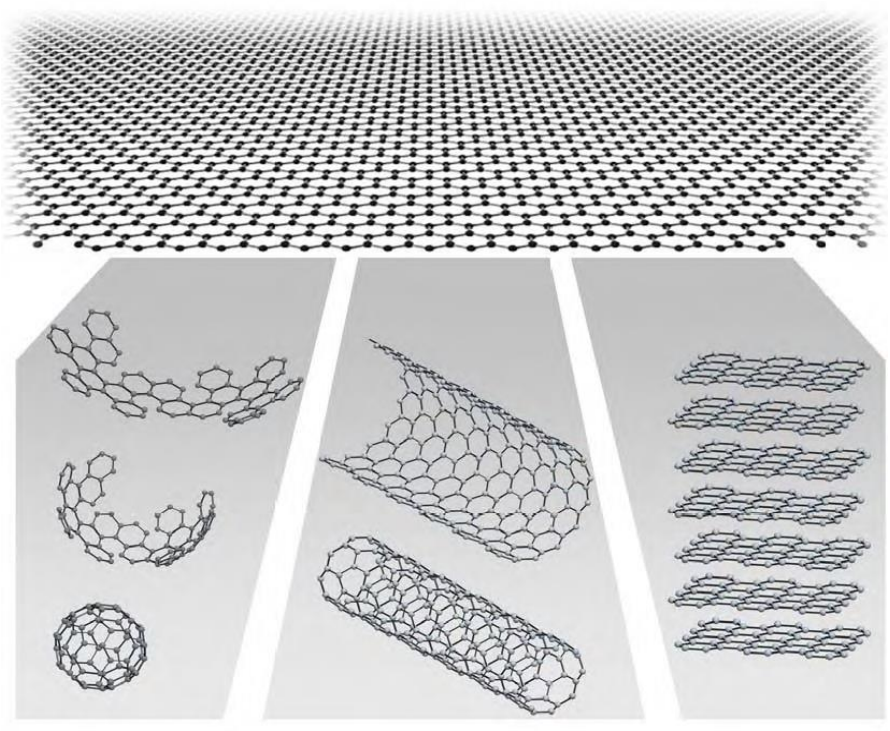

Figure 2-2: The honeycomb lattice of graphene which can be wrapped up into 0D fullerences, rolled into 1D nanotube or stacked into 3D graphite (Geim & Novoselov, 2007).

Recently graphene had emerged as an interesting material in a great number of applications. This can be attributed to its unique mechanical and electronic properties due to its long-range π-conjugation (Huang, 2010). Besides that, graphene provides excellent thermal conductivity, which is about 2000–4000 $W \cdot m^{-1} \cdot K^{-1}$ at room temperature (Pop *et al*., 2012).

In the electronic properties studies of graphene showed that it has the ability to tune the charge carriers from holes to electrons continuously which produced high electrical conductivity. This is due to it is a zero-overlap semimetal with each atom is connected to three other carbon atoms on the two dimensional plane, leaving one electron freely available in the third dimension for electronic conduction (Bunch, 2008). Tests have shown that the electronic mobility of graphene is very high, with previously reported results above 15,000 $cm^2 \cdot V^{-1} s^{-1}$ and theoretically potential limits of 200,000 $cm^2 \cdot V^{-1} s^{-1}$. This is because of the limitation of the scattering of graphene's acoustic photons (Geim & Novoselov, 2007).

Graphite is unique in that the elastic constants in the direction perpendicular are vastly different than the elastic constants along the basal plane. This was known for quite some time and was experimentally measured during the 1960s and 1970s. Due to the resurgent interest in graphene and few layer graphene structures, it is worthwhile to revisit this history of graphite (Kelly, 1981). Mechanical strength of graphene can be investigated by atomic force microscopy (AFM) (Zhu *et al.,* 2010). It shows a Young's modulus of 1.0 TPa and a fracture strength of 130 GPa by AFM (Zhu *et al.,* 2010).

More so, there are scarcities of studies into the use of graphene as a biosensor. Most of the prior graphene studies are related to its electronic properties and applications that are confined to gas and pH sensors (Hong *et al.,* 2009).

Table 2-3: Properties of Graphene.

Properties	Explanation	References
Structure	A hexagonal carbon lattice which tightly packed into a two dimensional (2D) atomic crystal.	Geim & Novoselov, 2007
Physical properties	It has high specific surface area, which is 2630 m^2/g for single-layer of graphene	-
Electronic conductivity	Above 15,000 $cm^2 \cdot V^{-1} s^{-1}$ and theoretically potential limits of 200,000 $cm^2 \cdot V^{-1} s^{-1}$.	Geim & Novoselov, 2007
Thermal conductivity	thermal conductivity of graphene at room temperature is about 2000–4000 W $m^{-1} K^{-1}$	Pop *et al*., 2012
Mechanical properties	Graphene shows a Young's modulus of 1.0 TPa and fracture strength of 130 GPa by AFM.	Zhu *et al.*, 2010

2.2.3 Application of Graphene

Due to the unique features of graphene, it had been employed in different fields. Thus graphene and its derivatives are expected to find applications in many fields such as nanoelectronic devices, chemical and biological sensors, energy storage and biomedical fields which have been summarized in Table 2.2-4.

Table 2-4: Applications of Graphene in Different Fields.

	Application		References
Graphene	Electronic nanodevices	Field effect transistors	Novoselov *et al*., 2004
		Transparent conductive films	Kim *et al.,* 2009
	Energy storage device	Li-ion capacitors	Paek *et al.*, 2009
		Ultra Capacitors	Stoller *et al.*, 2008
		Fuel cell and solar cells	Wu *et al.*, 2008
	Sensors	Electrochemical sensors	Schedin *et al.*, 2007
		Biosensors	Kuila *et al.*, 2011
	Biomedical engineering	Gene delivery	Park *et al.*, 2006
		Drug delivery	Liu *et al.*, 2008
		Tissue engineering	Fan *et al.*, 2010
		Cancer therapy	Liu *et al.*, 2008

2.3 Biosensor

2.3.1 Principle of Biosensor

Include Biosensor is a device employed for the detection of an analyte which combines a biological component with a physicochemical detector component (Sadana & Sadana, 2011). A working biosensor is comprised of three sections *viz*. the sensitive biological element, the transducer or detector element and also the associated electronics or signal processors (Chaplin & Bucke, 1990). The analytical devices consist of a biological recognition element directly interfaced to a signal transducer for correlating the concentration of an analyte to a measurable response (Li C. , 2010). The first biosensor, an oxygen electrode was developed by Professor Leland C. Clark in 1956 (Palchetti & Mascini, 2010). Today, this industry is a billion dollar industry. Even recent survey by Global Industry Analysts, Inc. indicates that global chemical biosensor market will touch $17.3 billion by year 2015. This is largely driven by the potential of developing new product application and furthering the usages and functions of current biosensors (Global Industry Analysts, 2010).

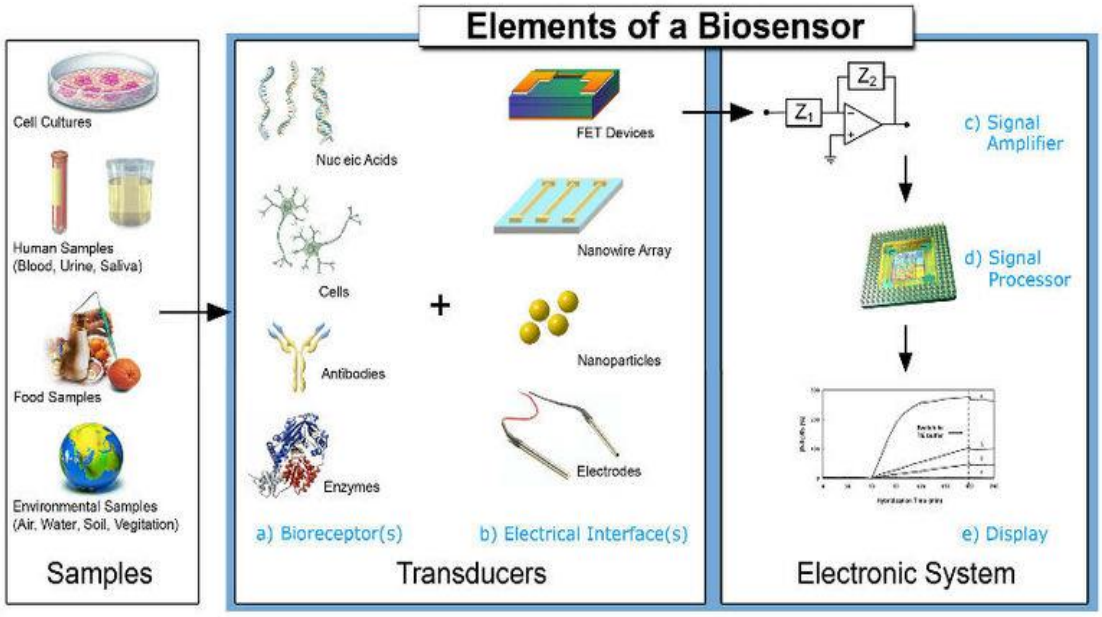

Figure 2-3: The working principle and components of biosensor (Grieshaber *et al.*, 2008).

Biosensors can be divided according to their analytes and detection mode (Joshi, 2006). Graphene-based enzymatic electrodes are based on the direct electrochemistry of enzymes involves in direct electron transfer between the electrode and the active center of the enzymes without the participation of the mediators or others reagents (Yao & Shiu, 2008; Shao *et al.*, 2009; Gao *et al.*, 2014). There are various types of enzymatics electrodes made from graphene based biosensor i.e. glucose sensor, NADH biosensor, haemoglobin biosensor, HRP sensor, catechol biosensor and cholesterol biosensor.

Table 2-5: Type of graphene based biosensor and detected element (Kuila et al., 2011).

Sensor Type	Detected element	References
Glucose biosensor	Glucose	Fan *et al.*, 2010
Cholesterol oxidase	Cholesterol	Dey & Raj, 2010
NADH	NADH	Keeley *et al.*, 2011
HRP	H_2O_2	Zhou *et al.*, 2010

2.3.2 Glucose Oxidase Biosensor

In 1962 Clark and Lyons of the Cincinnati Children's hospital first developed the device of glucose enzyme electrodes. Their first glucoseenzyme electrode relied on a thin layer of glucose oxidase (GOD) entrapped over an oxygen electrode via a semipermeable dialysis membrane. Measurements were made based on the monitoring of the oxygen consumed by the enzyme-catalyzed reaction:

$$glucose + O_2 \xrightarrow{GOD} gluconic\ acid + H_2O_2 \qquad (4)$$

A negative potential was applied to the platinum cathode for a reductive detection of the oxygen consumption.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \qquad (5)$$

This glucose enzyme electrodes started the entire field of biosensor. To measure the glucose concentration, 3 different transducers can be used. The first type is oxygen sensor that measures oxygen concentration and converts oxygen concentration into electrical current.Second type is pH sensor that measures the acid (gluconic acid) production and

converts pH change into voltage change. The third type is peroxide sensor that measures H_2O_2 concentration and converts peroxidase concentration into an electrical current.

Yang *et al.* (2006) have developed a glucose biosensor using a platinum nanowire nanoelectrode array (NEA). The authors used nanowires to fabricate a biosensor array. Their biosensor was able to determine glucose concentration in the range of 10^{-6} to 3×10^{-2} M. They claimed that their biosensor has a high efficiency of signal transduction and is able to determine glucose concentration in real blood samples. Moreover, their nanostructing process not only has increased the surface area and the number of electroactive sities, it also extended the upper detection limit. Glucose oxidase was used in their research which is stable when adsorbed onto the electrode surface.

Significantly, the application of graphene in highly sensitive and cost-effective biosensor can be developed in this field (Hansen *et al.*, 2006). Noble nanoparticles (NPs) such as platinum exhibit electrocatalytic behavior to H_2O_2 and have been widely used for sensing application (Choi *et al*., 2011). Chitosan with abundant amino groups exhibits good biocompatibility (Liu *et al.*, 2005) and excellent film-forming ability originating from its protonantion and solubility in slightly acidic solution and stability from insolubility in solution with pH over pK_a (6.3) (Denuziere *et al.*, 1998). These characteristics is suitable to immobilize bioactive molecules and to construct biosensor. Glucose oxidase will then be immobilized to form a bionanocomposite film. Finally, all the components will be combined to fabricate a graphene based glucose biosensor code-named reduced graphene oxide (RGO)-glucose oxidase (GOx).